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13. ABSTRACT ***(Maximum 200 Words)***

Over-expression of HER-2/neu has been linked to poorer prognosis and reduced survival in breast cancer patients. The basis for this association is likely multifactorial and includes therapeutic resistance, such as resistance to Taxol (paclitaxel), widely used in many chemotherapeutic regimens for this disease. We have recently reported that a paclitaxel copolymer, poly(L-glutamic acid)-paclitaxel (PGA-TXL), is active against Taxol-resistant human ovarian xenograft models *in vivo* (Auzenne *et al.* Superior therapeutic profile of poly-L-glutamic acid-paclitaxel copolymer compared to Taxol in xenogeneic compartmental models of human ovarian carcinoma. *Clin Cancer Res* 8: 573-581, 2002). We therefore evaluated PGA-TXL in human HER-2/neu over-expressing (MDA-361) and low/null-expressing (MDA-231) breast adenocarcinoma orthotopic xenograft models. PGA-TXL (20-37% paclitaxel, w/w) was administered as a single dose of 180 mg (paclitaxel equivalents)/kg i.p., near its MTD. Drug was administered either one week after tumor implantation or once tumors were 4-5 mm in diameter. Either regimen resulted in growth inhibition of 361 tumors, and even some apparent cures with early treatment ($p < 0.04$). An on-going experiment with the 231 model demonstrates a similar initial pattern. We conclude that systemic treatment with PGA-TXL is active against human breast adenocarcinoma orthotopic xenograft models despite over–expression of HER-2/neu.

14. SUBJECT TERMS
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INTRODUCTION

Taxol has proven to be a valuable addition to the chemotherapeutic regimens that can be offered to breast cancer patients; however, as with other drugs, evidence for resistance to Taxol has emerged. Among these resistance mechanisms is the P-gp 170 membrane-associated drug-efflux pump, encoded by *MDR1*, and over-expression of the oncogene, HER-2/neu. Both of these mechanisms are widely associated with breast cancer. Strategies to address Taxol-resistance include its combination with other chemotherapeutic agents and dose-intensification. However, in recent randomized clinical trials, the latter has proven to be a case of diminishing returns, with little meaningful clinical benefit at the price of severe toxicities. Therefore, new agents and strategies are urgently needed to address Taxol-resistant breast cancer.

One approach to overcoming drug resistance is the use of drug copolymers. These high molecular weight conjugates can be actively transported to the endosome, and are then cleaved to release free drug at this organelle. For DNA-targeting drugs, this may afford superior nuclear access compared to import via diffusion as occurs with free drug. Further, it restricts the gradient of export of conjugate-released drug via membrane-localized drug efflux mechanisms that are clearly operant on free drug. In vivo, other considerations may be more relevant, including distribution to tumor vs. normal tissue. High molecular weight drug copolymers may, on the one hand, 1) restrict diffusion-controlled uptake by normal tissues that occurs with free drug; but, on the other, they may 2) enhance extravasation across the abnormal tumor endothelium, thereby enhancing tumor localization compared to free drug. The Taxol copolymer employed in these studies has shown both reduced toxicity and greater tumor localization in other animal models, thereby fulfilling two expectations of copolymer behavior. To date, it has also demonstrated reduced toxicity and greater ease of administration compared to Taxol in the clinic, and has shown activity in patients with Taxol-refractory tumors. In this proposal, we will establish the toxicity, pharmacokinetics and anti-tumor efficacy of this Taxol copolymer in human breast adenocarcinoma models in nude mice; these models of P-gp- and HER-2/neu-mediated resistance will test the potency of the copolymer against resistance mechanisms that are operant against Taxol itself.

BODY

Task 1 Mechanistic Studies: Effects on Cell Cycle Distribution/Apoptosis and RAF-1 Kinase Activation

a) Conduct cell-cycle (PI staining) and apoptosis assays (TUNEL and hypodiploidy) on human breast adenocarcinoma cell lines (P-gp and HER-2/neu models) to be used in Task 4 to establish responses to Taxol and PGA-TXL in vivo

b) Using these cell lines and the doses established as relevant to previous endpoints, determine role of Raf-1 kinase pathway in these responses

Studies related to this task have not yet been initiated.

Task 2 Pharmacokinetics: Cellular and IP Administration

a) Establish parameters of cellular uptake and fate of paclitaxel and PGA-TXL, using compounds ^{3}H-labeled in paclitaxel moiety or in PGA backbone; determine extent and site of PGA-TXL esterolysis to paclitaxel

b) Establish pharmacokinetic parameters for peritoneal clearance of paclitaxel and PGA-TXL following i.p. administration; determine parameters for resultant plasma levels compared to i.v. administration; determine extent and site of PGA-TXL esterolysis to paclitaxel

Studies related to this task have not yet been initiated.

Task 3 Toxicity Studies: Single- and Multiple-Dose IP and IV MTDs

a) Determine single-dose i.v. and i.p. MTD for PGA-TXL in nude mice

Task 4 Efficacy Studies: Her-2/neu- and P-gp-Mediated MDR Models
HER-2/neu-mediated MDR

a) Establish tumor responses and effects on survival of Taxol or PGA-TXL administered at single- or multiple-dose MTDs to nude mouse models of HER-2/neu high and basal expressing human breast adenocarcinomas

Activities relevant to these two Tasks are presented below.

Poly(L-glutamic acid)-paclitaxel (PGA-TXL) was prepared by carbodiimide-mediated coupling of paclitaxel and poly(L-glutamic acid). Formulations of the final product contained ~20% or ~37% paclitaxel (w/w), with a PGA backbone of ~30-40 k Da.

MDA-MB-361 human breast adenocarcinoma cells were obtained from the ATCC and were cultured exactly according to the ATCC-defined conditions and using their specific recommended serum. The cells were maintained in Liebowitz L-15 medium in the absence of CO_2. This allowed retention of original cellular morphology and growth pattern in vitro, albeit with a long doubling time (~7 days). Cells were finally trypsinized and adjusted to an inoculum cell number of 4-6 X 10^6 viable cells.

MDA-361 cells were implanted under asceptic conditions in the mammary fat pad of 5-8 week old female nude mice. After one week, one group of inoculated mice was treated with PGA-TXL. The formulation was injected i.p. in 100-200 microliters volume of PBS. A single dose level of 180 mg/kg was administered one time only. Controls were given saline. Another group of mice was treated with the same regimen of PGA-TXL 38 days later when perpendicular tumor diameters were ~ 4 ± 0.5 mm by caliper measurement. Tumor outgrowth was evaluated by caliper measurement of perpendicular tumor diameters in treated and control groups; the product of those diameters was plotted vs. the day of measurement.

Early treatment (one week after tumor implantation) with a single-dose of PGA-TXL resulted in substantial tumor growth control and even apparent cure of some mice (Fig. 1). On Day 98, tumor areas of control mice averaged 98.1 ± 23.0 mm^2 (mean ± SEM), whereas tumor areas of the early PGA-TXL treatment group averaged 10.2 ± 5.9 mm^2 ($p < 0.04$ vs. control). The tumor growth curve following this single PGA-TXL treatment was suggestive of prolonged stasis, with anti-tumor effects evident for as long as ~ 10 weeks after this treatment. Only slight further growth occurred in the next 30 days, reflecting a much slower growth rate than for controls.

When administration was initiated at the later timepoint (Day 38), at which time tumor diameters for controls averaged ~ 3.9 mm and those for the new treatment group, ~ 4.4 mm, treatment with PGA-TXL was efficacious, but less so than with early treatment. For example, no apparent cures were achieved with PGA-TXL administered at this later

timepoint. In addition, when measured on Day 98, tumor areas of the late PGA-TXL treatment group averaged 50.6 ± 23.8 mm^2, compared to 98.1 ± 23.0 mm^2 for the controls ($p < 0.28$; Fig. 1). In part, the lack of statistical significance could be attributed to the small group size, something that will be addressed in the next, more definitive experiments. The tumor growth curve following this single treatment was again suggestive of stasis at the initial tumor volume, with effects evident for as long as ~ 25-30 days after this treatment (Fig. 1). Further growth occurred in the next 30-40 days, more aggressive than observed when tumors were treated early, but still reflecting a somewhat slower rate (lower slope) than for controls.

KEY RESEARCH ACCOMPLISHMENTS

- The key results from this study indicate that even single-dose PGA-TXL, in two different treatment regimens, is active against an orthotopically-implanted human Her-2/neu over-expressing breast tumor model. Activity was superior in the early treatment group, suggesting a dependence on tumor burden.

REPORTABLE OUTCOMES

Three abstracts/presentations have arisen from this work to date, and following verification and extension to statistical significance of these studies, we will prepare a manuscript.

1) The following abstract was submitted and accepted to the 6th US-Japan Symposium on Drug Delivery Systems, held in December, 2001 in Maui, HI. It was presented as a poster as well as being selected for presentation in a workshop.

PACLITAXEL COPOLYMER TO ADDRESS TAXOL RESISTANCE

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We have evaluated a paclitaxel-poly(L-Glu) copolymer in human tumor/nude mouse orthotopic xenograft models which either reflect resistance to Taxol (HEY/ovarian) or over-express HER-2/neu (MDA-361/breast). Early treatment (Day 2 HEY) with MTD Taxol achieved some improvement in survival, but was not curative. However, treatment with copolymer markedly improved survival and some apparent cures were observed. The higher tumor

burden at Day 7 rendered this model resistant to MTD Taxol, but still responsive to copolymer. Similarly, early treatment (Day 7) of the 361 breast model with paclitaxel copolymer resulted in substantial tumor growth delay, regression, or even apparent cure. When administered later, the copolymer still caused tumor growth delay, but no cures were observed. We conclude that formulation of paclitaxel with this poly(L-Glu) backbone substantially enhanced its potency, and rendered it active in two highly drug-resistant models. Supported in part by DOD grants BC980420, BC991113 and OC000036 (JK).

2) The following abstract was presented as a poster at the Era of Hope Meeting, sponsored by the DOD Breast Cancer Research Program, in Orlando, FL, September 25th-28th, 2002.

LIPOSOMAL-DIMETHYL-SPHINGOSINE AND PACLITAXEL COPOLYMER ARE ACTIVE AGAINST HER-2/NEU OVER-EXPRESSING HUMAN BREAST ADENOCARCINOMA ORTHOTOPIC XENOGRAFT MODEL

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Over-expression of HER-2/neu has been linked to poorer prognosis and survival in breast cancer patients. The basis for this association likely includes therapeutic resistance, including resistance to Taxol (paclitaxel), widely used in many chemotherapeutic regimens for this disease. We have recently observed that certain sphingolipids, either as free lipids or as constituents of liposomes, induce apoptosis in vitro in tumor cells despite the over-expression of Her-2/neu. Further, we have reported that a paclitaxel copolymer, paclitaxel-poly(L-glutamic acid) (PGA-TXL), is active against Taxol-resistant tumors in vivo.

We therefore evaluated liposomal-dimethyl-sphingosine (L-DMSP) and PGA-TXL in a human HER-2/neu over-expressing breast adenocarcinoma (MDA-361) orthotopic xenograft model. Tumor cells (4-6 X 10^6) were implanted in the mammary fat pad of 5-8 week old female nude mice. Mice were treated i.p. either one-week later or when tumors grew to 5-6 mm diameter.

Early treatment with a multiple-dose regimen of L-DMSP (4.5 mg DMSP per dose; 20 mole percent of a small unilamellar vesicle formulation), caused a delay in or reduced subsequent tumor growth, but was not curative. However, early treatment with a single-dose of PGA-TXL (180 mg/kg paclitaxel equivalents), also one week after tumor implantation, resulted in substantial tumor growth delay, regression, or even apparent cure in two of four mice (control tumor areas at 10 weeks post-implant = 44 ± 21.2 mm^2 ; treated group areas = 6 ± 6.0 mm^2). When administered at the later timepoint to another group of animals, PGA-TXL still caused tumor growth delay, but no cures were observed (treated group areas = 24 ± 15.3 mm^2); nor did administration of L-DMSp at this time appear to be efficacious.

We conclude that DMSP as a liposomal formulation has some efficacy against this HER-2/neu over-expressing model when the tumor burden is low. Formulation of paclitaxel with the poly(L-glutamic acid) backbone substantially reduced its toxicity, enhanced its potency, and rendered it active against this HER-2/neu over-expressing breast adenocarcinoma model.

Supported by U.S. Army Medical Research and Material Command under DAMD17-99-1-9265 and DAMD17-00-1-0313.

3) The following abstract, submitted online, was accepted for presentation as a poster at the AACR/EORTC Meeting to be held in Frankfurt, Germany on November 19-23, 2002.

Therapeutic resistance to Taxol is a major issue in a number of cancers, particularly breast and ovarian carcinoma. This resistance is multifactorial, including P-gp170-linked MDR and over-expression of HER-2/neu. We evaluated the efficacy of a paclitaxel-poly(L-Glu) copolymer (PGA-TXL) in a human ovarian carcinoma orthotopic xenograft model which reflects resistance to Taxol (HEY); we also evaluated PGA-TXL as well as a liposomal (SUV) formulation of dimethyl-sphingosine (L-DMSP; which induces apoptosis in a broad spectrum of tumor cell lines *in vitro*) in an orthotopic human breast adenocarcinoma model that over-expresses HER-2/neu (MDA-361). In the ovarian model, early treatment (Day 2 post-implantation) with multiple-dose MTD Taxol (10 mg/kg) i.p. achieved slight improvement in survival, but was not curative. However, treatment with a single dose (180 mg/kg, paclitaxel equivalents) of PGA-TXL i.p. markedly improved survival and induced some apparent cures. The higher tumor burden present on Day 7 rendered this model resistant to MTD Taxol administration at this time, but still responsive to PGA-TXL. For the breast model, treatment on Day 7, before tumors were palpable, with PGA-TXL resulted in subsequent tumor growth delay, regression, or even apparent cure. Treatment at this time with a multiple-dose regimen of L-DMSP (4.5 mg DMSP/dose) i.p., caused a delay in or reduced subsequent tumor growth, but was not curative. When administered later after tumors grew to 5-6 mm diameter, PGA-TXL still caused tumor growth delay, but no cures were observed; administration of L-DMSP at this later time was not efficacious We conclude that formulation of paclitaxel with this poly(L-Glu) backbone substantially enhanced its potency, and rendered it active in drug-resistant ovarian and breast models. Further, we conclude that DMSP as a liposomal formulation has some efficacy against this HER-2/neu over-expressing breast model: however, only when the tumor burden is low. (Supported in part by DOD grants BC980420, BC991113 and OC000036 to JK).

CONCLUSIONS

HER-2/neu over-expression in breast cancer portends an aggressive clinical course and greater resistance to certain therapeutic regimens, including those involving taxanes. Although the advent of Herceptin has brought new opportunities for more effective and targeted therapy for women with this marker, other approaches must also be exploited. The use of a drug copolymer strategy for paclitaxel (Taxol) based on a poly(L-glutamic acid; PGA) backbone has proven in pre-clinical and clinical studies to reduce the toxicity of paclitaxel. Importantly, activity of PGA-TXL in Taxol-resistance settings has been observed, as well.

The key inference: our pre-clinical studies suggest that among the patients who could be considered for trials with PGA-TXL are those with tumors over-expressing HER-2/neu and refractory to conventional taxanes.

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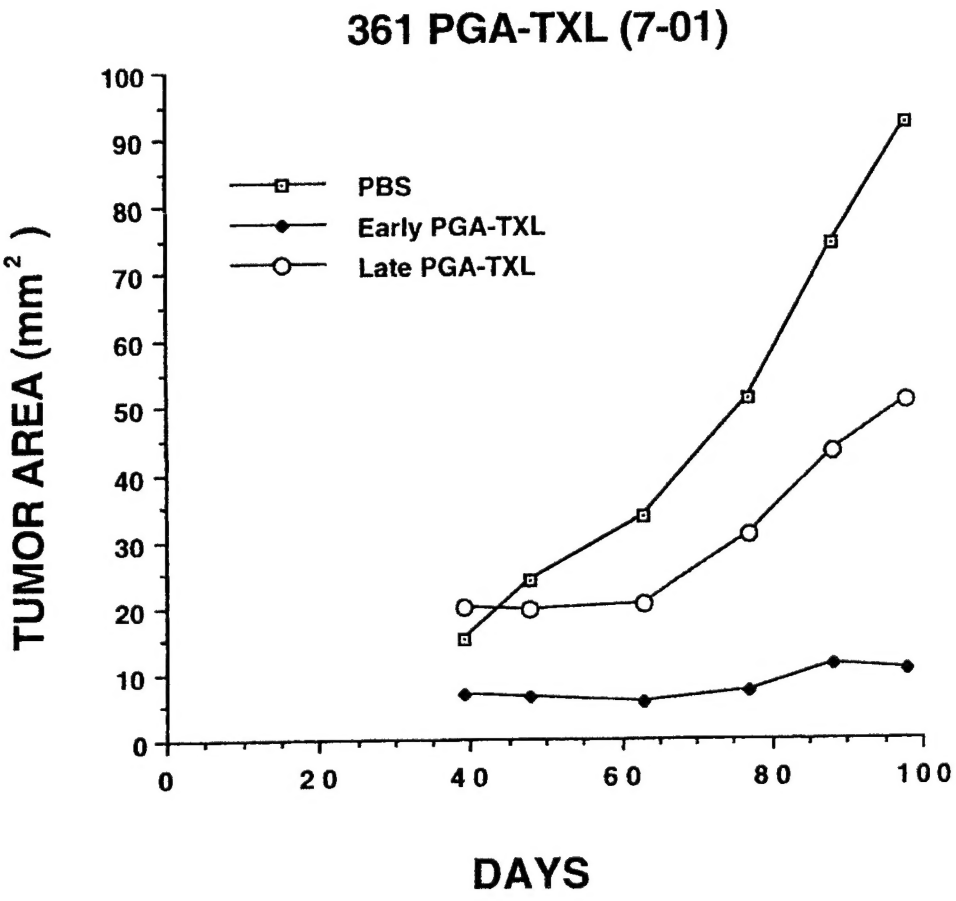
APPENDIX

Figure 1 Responses of 361 model to PGA-TXL

PGA-TXL was administered i.p. on Day 7 (early treatment) or on Day 38 (late treatment).


361 PGA-TXL (7-01)
TUMOR AREA (mm^2)
DAYS
PBS
Early PGA-TXL
Late PGA-TXL
0
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100